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The upper surface of an *Escherichia coli* swarm is stationary

R. Zhang^a, L. Turner^a & H.C. Berg^{a,b,1}

^aRowland Institute at Harvard, Cambridge, MA 02142

^bDepartment of Molecular and Cellular Biology, Harvard University, Cambridge,
MA 02138

Corresponding author: Howard C. Berg, Department of Molecular and Cellular
Biology, Harvard University, Biological Laboratories, 16 Divinity Avenue,
Cambridge, MA 02138, tel 617-495-0924, fax 617-496-1114, e-mail

hberg@mcb.harvard.edu

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¹To whom correspondence should be addressed: E-mail:

hberg@mcb.harvard.edu.

When grown in a rich medium on agar, many bacteria elongate, produce more flagella, and swim in a thin film of fluid over the agar surface in swirling packs. Cells that spread in this way are said to swarm. The agar is a solid gel, with pores smaller than the bacteria, so the swarm/agar interface is fixed. Here we show, in experiments with *Escherichia coli*, that the swarm/air interface also is fixed. We deposited MgO smoke particles on the top surface of an *E. coli* swarm near its advancing edge, where cells move in a single layer, and then followed the motion of the particles by dark-field microscopy and the motion of the underlying cells by phase-contrast microscopy. Remarkably, the smoke particles remained fixed (diffusing only a few μm), while the swarming cells streamed past underneath. The diffusion coefficients of the smoke particles were smaller over the virgin agar ahead of the swarm than over the swarm itself. Changes between these two modes of behavior were evident within 10 to 20 μm of the swarm edge, indicating an increase in depth of the fluid in advance of the swarm. The only plausible way that the swarm/air interface can be fixed is that it is covered by a surfactant monolayer pinned at its edges. When a swarm is exposed to air, such a monolayer can markedly reduce water loss. When cells invade tissue, the ability to move rapidly between closely-opposed fixed surfaces is a useful trait.

bacterial motility | surfactant monolayer | magnesium oxide smoke

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The ability of cells of *Escherichia coli* to swarm over an agar surface depends upon the structure of the agar: Eiken agar works well but Difco agar does not, presumably because Eiken agar is more wettable (1, 2). When given the choice in a microchannel of swimming near agar or polydimethylsiloxane (PDMS) made hydrophilic by exposure to an oxygen plasma, the cells prefer agar (3). When swimming over a glass surface in a layer of fluid much thicker than the bacteria, cells spiral to the right (4), because the cell bodies, which are in front, roll clockwise over the surface, while the flagellar bundles, which push from behind, roll counterclockwise. The torque resulting from this couple causes cells to veer to the right. When tracking cells in *E. coli* swarms, we found that cells prefer to swim straight ahead, curving to the left about as much as to the right. This behavior makes sense if the upper surface of an *E. coli* swarm is stationary, because the torques generated at the upper and lower surfaces are of opposite sign and will cancel. Here, we prove that the air/water interface is stationary by following the motion of particles of MgO smoke allowed to settle on the surface of a swarm. The geometry of the experiment is sketched in Fig. 1, which shows two cells at the advancing edge of a swarm (right) and two smoke particles at the air/water interface (left and right). As we shall show, cells stream underneath these particles without seriously perturbing their motion; the particles continue to move at random (diffuse) within a small region as the cells swim by. The diffusion coefficient of a particle in a lipid membrane over an aqueous film is

known to increase with the thickness of the film (5), so changes in the diffusion coefficient of a given particle plotted as a function of the distance from the edge of an advancing swarm allowed us to gauge changes in the depth of the fluid between the air/water interface and the surface of the agar: the fluid is shallow over the virgin agar and deeper at the swarm, with a region of increasing depth in between.

Results

Small Particles Float on the Upper Swarm Surface. We tried an array of powders, such as alumina or diatomaceous earth or talc, but their particles tended to aggregate and get stuck in the agar at the bottom of the swarm. Then we hit upon particles of smoke that could be generated by burning magnesium ribbon. Large particles of MgO smoke tended to sink into the swarm and get stuck in the agar at the bottom or be pushed around by the moving cells, but small particles ($\sim 0.2\ \mu\text{m}$ in diameter) remained on the upper surface. Figure 2 shows images from a video clip of a smoke particle sitting on the surface of a swarm. The particle continued to move at random within a small region near the center of the field, while swarm cells streamed past underneath. Panel *A* shows the particle before the swarm cells arrived, panel *B* shows the particle as the swarm cells arrived, and panels *C* and *D* show the particle after a number of cells had passed underneath. The particle remained at nearly the same position, but it was free to diffuse, as shown by the red trace in panel *D*. More than 30 small smoke particles were observed on 3 swarm plates, and all behaved in a similar

manner. By adjusting the focus, it was clear that the particles were floating above the cells, at the air/water interface. We conclude that this interface is stationary: it does not move with the swarming cells.

Small Particles Diffuse. The mean-squared displacements of two smoke particles are shown as a function of time in Fig. 3. The diffusion is two-dimensional, with $\langle r^2 \rangle = 4Dt$, where r is the displacement and D is the diffusion coefficient, which depends upon the size of the particle. For every particle analyzed in this way, the slopes of these plots were larger when the particle was over the swarm than when the particle was over the virgin agar, by factors ranging from 1.3 to 3.2. Fig. 4 shows the diffusion coefficient of one particle as a function of its distance from a swarm front. When the particle was above the agar but some distance from the advancing swarm, its diffusion coefficient was relatively small and remained constant. As the cells approached, the diffusion coefficient increased. When the particle was above the swarm, the diffusion coefficient was relatively large (and in most cases, approximately constant). These results show that particle diffusion can be used to probe the thickness of the layer of fluid in front of an advancing swarm.

Discussion

The Air/water Interface is Stationary. The failure of small MgO smoke particles to be swept along with cells of a swarm (Fig. 2) indicates that the air/water (or air/fluid) interface is stationary. The ability of the particles to diffuse locally (Figs. 3, 4) indicates that this interface is fluid. In all likelihood, the

interface is covered by a monolayer of surfactant that spreads until it reaches the edges of the plate, which prevent it from moving farther. Monolayers of this kind are strong enough to resist the viscous drag of fluid generated by the motion of swimming cells. Langmuir (6) found that such monolayers can support surface pressures in excess of 50 dynes/cm (0.05 N/m). The surface pressure generated by a 10-cm long carpet of cells moving uniformly between two parallel plates 1 μm apart at an average rate of 5 $\mu\text{m/s}$, assuming a parabolic flow profile, is about 3 dynes/cm; see pp. 53-54 of (7). This is an over-estimate, since swarms do not move in such a concerted fashion. If the air/water interface is stationary, it is clear why the average curvature of tracks of swarming cells are small -- torques in one direction generated at the agar/water interface are balanced by torques in the other direction generated at the air/water interface.

The Layer of Fluid Above the Agar is Thicker Near the Swarm. The diffusion coefficient of a particle embedded in a lipid membrane over a thin layer of fluid depends upon the viscosities and thicknesses of the membrane and the fluid. In general, the diffusion coefficient increases with the thickness of the fluid (which in our case is an aqueous medium), because the viscous drag on the particle is smaller when the viscous shear in the fluid is smaller (when the fluid is thicker). This problem was posed by Saffman & Delbrück (8) for a disk in a membrane immersed in an infinite medium, extended by Evans & Sackmann (9) for a disk in a membrane over a fluid film that is very thin, and then generalized by Stone & Ajdari (5) for a disk in a membrane over a fluid film of finite thickness. In their Fig. 2, Stone and Ajdari plot the drag coefficient for a disk (in dimensionless

form) as a function of H/R , where H is the thickness of the fluid and R is the radius of the disk. They show a series of curves each characterized by a dimensionless parameter $\Lambda = \eta R / \eta_m h$, where h is the thickness of the membrane (the surfactant layer), η is the viscosity of the fluid, and η_m is the viscosity of the membrane. For our problem, reasonable values for these parameters are $R = 0.1 \mu\text{m}$, $h = 40 \text{ nm}$, and $\eta/\eta_m = 0.01$, yielding $\Lambda \sim 0.03$. Assuming a thickness of fluid at the edge of the swarm $\sim 1 \mu\text{m}$, $H/R \sim 10$. If we move along the $\Lambda = 0.03$ curve from $H/R = 10$ to $H/R = 1$, the drag increases by a factor of about 1.6. This is in the range observed in our experiments. So our data suggest that the fluid film is roughly 10 times thicker at the edge of the swarm than it is over the virgin agar. The absolute values of the diffusion coefficients indicated in Fig. 4 are about an order of magnitude smaller than $2.2 \mu\text{m}^2/\text{s}$, the value expected for a sphere of radius $0.1 \mu\text{m}$ diffusing in water. The smoke particle encounters higher drag because the surfactant layer is more viscous than water, and the viscous shear in the thin layer of fluid beneath it is larger than the shear in an infinite medium.

The Source of the Surfactant Is Not Known. In an earlier study (10), we found that swarming cells of *Salmonella* produced a surfactant that reduced the contact angles of drops of fluid that were harvested from cell lawns (cells spread uniformly over agar) and placed on a hydrophobic surface (freshly prepared PDMS). Drops harvested from certain non-swarming mutants behaved in the same way; although, their cell lawns were relatively dry. We concluded that differences in dryness were due to another factor, missing in the mutants, an

osmotic agent that draws water out of the underlying agar. Presumably, *E. coli* produces similar materials, which might include a surfactant that finds its way to the air/water interface. However, surfactants could come from the Eiken agar, or even from our growth medium, which is more complex than the medium used for *Salmonella*. Secretion of surfactants has not been demonstrated for *E. coli*; although, surfactants are well known for other swarming bacteria: see the references cited in (10). It is generally assumed that these surfactants promote wetting of solid substrates. Another important function might be to keep swarms wet. Close-packed monolayers of long-chain compounds, such as hexadecanol, are known to reduce water loss.

***E. coli* can Swim in Thin Films or in Narrow Constrictions.** Wu and Libchaber (11) studied the motion of *E. coli* and relatively large latex beads (up to 10 μm in diameter) in a freely suspended soap film. The bacteria were found to move the beads in a superdiffusive manner. These films were thought to be as thick as the beads, and hence substantially thicker than the films dealt with here. The smoke particles that we tracked on the surface of the swarm exhibited normal diffusion, not superdiffusion, with diffusion coefficients that were relatively small (as noted above). Others have studied *E. coli* swimming in thin glass channels (12, 13). Our experiments demonstrate the ability of such bacteria to swarm vigorously between two closely-opposed fixed surfaces. This must afford cells a competitive advantage, e.g, when invading tissue.

Materials and Methods

Bacteria and Swarm Plates. We used *E. coli* strain HCB1668, a FliC S353C derivative of wild-type strain AW405 (HCB1). Its construction, maintenance, and growth are described elsewhere (Turner et al., in preparation). Polystyrene petri plates (150 x 15 mm) were filled with 25 ml swarm agar (0.45% Eiken agar in 1% Bacto peptone, 0.3% beef extract, 0.5% NaCl, and 0.5% L-arabinose), swirled gently to ensure complete wetting, and then cooled 15 min (without a lid) inside a large plexiglass box. The plates were inoculated with 2- μ l drops of cells grown to saturation in LB-broth (1% Bacto tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.5) and diluted 10^{-5} . The plates were dried for another 5 min, covered, and incubated for ~16 h at 30°C in a humid incubator.

Smoke Particles on Swarm Surface. MgO is hydrophobic and has a refractive index of ~1.7, suitable for imaging. Although smoke particle size is broadly distributed (14), a sub-population is very small (<0.2 μ m). To prepare particles of this size, the smoke from a piece of burning magnesium ribbon was collected with an inverted beaker (25 cm tall) that was allowed to stand for ~2 min, so that large particles could settle out. The beaker was placed over a swarm plate for ~5 min in a warm-water bath, which prevented the plate from cooling off or drying out. This procedure was repeated several times to give a conveniently large number of particles on the surface for imaging.

Imaging. The motions of particles and cells were monitored with a phase-contrast microscope equipped with an enclosed temperature-controlled stage (Nikon Optiphot, 20x 0.4 NA objective, 8x relay lens, 30°C) connected to a CCD camera and a digital tape recorder. The agar was sufficiently thin (~1.6 mm) that

phase-contrast illumination could be used from below. However, particles were hard to visualize when over cells, so dark-field illumination was added from the side, with a fiber-optic illuminator mounted $\sim 10^\circ$ from the horizontal and pointed at the spot below the microscope objective. The particles that we tracked were about $0.2\ \mu\text{m}$ in diameter, as judged by comparisons of their images with those of latex particles of known size, but this comparison was crude.

Particle Tracking. Particle positions were tracked with a program based upon an open-source MatLab package; see (http://www.rowland.harvard.edu/labs/bacteria/index_software.html). The locations of particles in each image were determined by selecting areas in which pixel values were above a specified threshold, removing noise by a series of simple morphology operations, and computing the x,y coordinates of the particle centroids. Searches for centroid positions were made within a $2\text{-}\mu\text{m}$ radius in successive frames. Particle tracks were superimposed onto the video images.

Computation of Diffusion Coefficients. To follow changes in the diffusion coefficient with time, a window of fixed width (4 s) was moved through the track, and the mean-square deviation was plotted for this time span, yielding a running value for D that changed from frame to frame. The time assigned to D in D vs. time plots was the time corresponding to the beginning of this moving time window. Time was converted to distance from the swarm edge by multiplying by the measured speed of displacement of this edge.

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FIGURE LEGENDS

Fig. 1. Two magnesium oxide smoke particles on the surface of an agar plate supporting an *E. coli* swarm, shown advancing from right to left, as indicated by the large arrow. One particle (left) is over virgin agar well ahead of the swarm; the other particle (right) is over the swarm. Two cells are shown (one truncated). They are rod-shaped, $\sim 1\ \mu\text{m}$ in diameter by $\sim 5\ \mu\text{m}$ long, and move in a thin layer of fluid (growth medium) at speeds of order $40\ \mu\text{m/s}$, more slowly at the edge of the swarm than farther behind. The fluid under the smoke particle at the left is shallower than the fluid under the smoke particle at the right. The fluid within the agar is more than a thousand times deeper ($\sim 1.6\ \text{mm}$).

Fig. 2. A smoke particle in a stationary air/water interface. The particle is shown at the center of each panel before (A), during (B), or after (C, D) the arrival of a swarm. The cells moved with speeds of $\sim 20\ \mu\text{m/s}$ in random directions near the edge of the swarm, while the swarm front drifted more slowly to the left at a speed of $\sim 1.7\ \mu\text{m/s}$. The smoke particle remained at nearly the same place but was free to diffuse, as shown by the red track in panel (D), which followed the centroid of the particle for 6.7 s. The field of view is $21.4\ \mu\text{m} \times 13.6\ \mu\text{m}$, and elapsed time is shown at the lower left-hand corner of each panel. For video movies of this and other smoke experiments, see http://www.rowland.harvard.edu/labs/bacteria/movies_swarmecoli.html.

Fig. 3. Diffusion of smoke particles. The mean-square displacements (MSD) of two particles are shown as a function of time (every third data point). The slopes

of these curves were larger for a given particle over a swarm than over virgin agar.

Fig. 4. Diffusion coefficient (D) of a particle as a function of its distance from a swarm front. (A) As the swarm approached the particle. (B) As the swarm moved beneath the particle. The depth of the fluid above the agar increased near the swarm's advancing edge. Changes in the diffusive behavior of particles were evident over a span of roughly 10 to 20 μm in front of advancing swarms.

References

1. Harshey RM & Matsuyama T (1994) Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. *Proc. Natl. Acad. Sci. USA* 91:8631-8635.
2. Harshey RM (1994) Bees aren't the only ones: swarming in Gram-negative bacteria. *Molec. Microbiol.* 13:389-394.
3. DiLuzio WR, *et al.* (2005) *Escherichia coli* swim on the right-hand side. *Nature* 435:1271-1274.
4. Lauga E, DiLuzio WR, Whitesides GM, & Stone HA (2006) Swimming in circles: motion of bacteria near solid boundaries. *Biophys. J.* 90:400-412.
5. Stone HA & Ajdari A (1998) Hydrodynamics of particles embedded in a flat surfactant layer overlying a subphase of finite depth. *J. Fluid Mech.* 369:151-173.

6. Langmuir I (1917) The constitution and fundamental properties of solids and liquids. II. Liquids. *J. Am. Chem. Soc.* 39:1848-1906.
7. Berg HC (1993) *Random Walks in Biology* (Princeton, Princeton, NJ).
8. Saffman PG & Delbrück M (1975) Brownian motion in biological membranes. *Proc. Natl. Acad. Sci. USA* 72:3111-3113.
9. Evans E & Sackman E (1988) Translational and rotational drag coefficients for a disk moving in a liquid membrane associated with a rigid substrate. *J. Fluid Mech.* 194:553-561.
10. Chen BG, Turner L, & Berg HC (2007) The wetting agent required for swarming in *Salmonella enterica* serovar Typhimurium is not a surfactant. *J. Bacteriol.* 189:8750-8753.
11. Wu X-L & Libchaber A (2000) Particle diffusion in a quasi-two-dimensional bacterial bath. *Phys. Rev. Lett.* 84:3017-3020.
12. Biondi SA, Quinn JA, & Goldfine H (1998) Random motility of swimming bacteria in restricted geometries. *AIChE J.* 44:1923-1929.
13. Männik J, Driessen R, Galadja P, Keymer JE, & Dekker C (2009) Bacterial growth and motility in sub-micron constrictions. *Proc. Natl. Acad. Sci. USA* 106:14861-14866.
14. Jokes CF, Segall RL, Smart RSC, & Turner PS (1980) Size distribution of MgO smoke particles. *Philos. Mag.* A42:267-270.

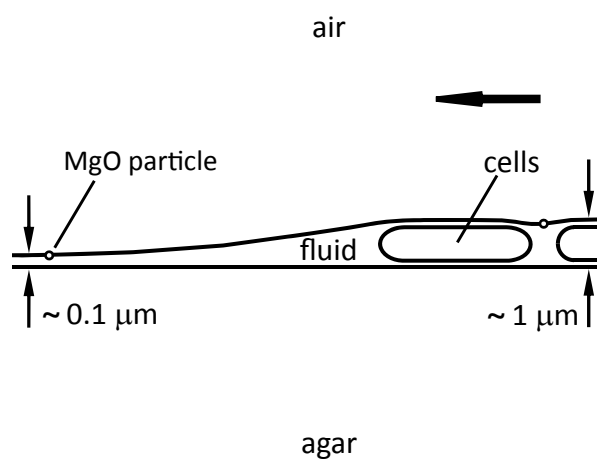


Fig. 1

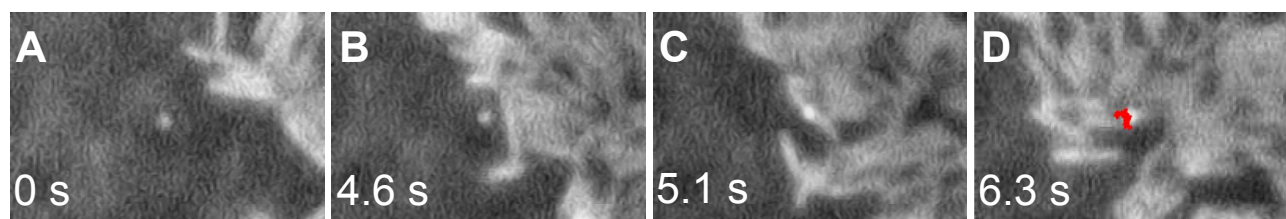


Fig. 2

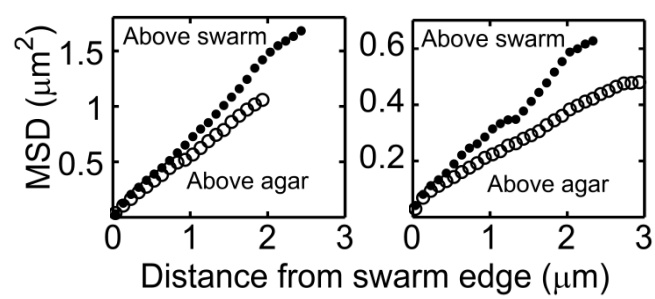


Fig. 3

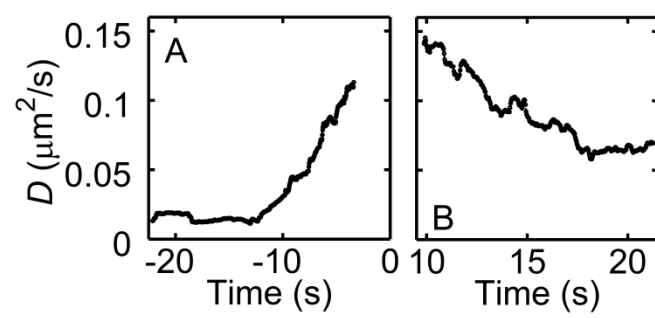


Fig. 4